Risk factor	Cases	Controls	Protective efficacy of MCCV	*Controls from risk factor study
>1 intimate kissing contact	3/25 (12%)	2/12 (17%)	94%	21/132 (16%)
Shared a bedroom with >1 person	14/25 (56%)	5/12 (42%)	94%	70/134 (52%)
Regular smoker	8/25 (32%)	3/12 (25%)	96%	45/134 (34%)
>1 friend or relative who smokes	17/24 (71%)	7/12 (58%)	94%	87/133 (65%)
Parental home ownership	18/23 (78%)	8/12 (67%)	95%	107/133 (80%)
Preceding illness	10/20 (50%)	5/10 (50%)	92%	40/134 (30%)

*Included for comparison.

Table 2: **Protective effectiveness of meningococcal C conjugate vaccine (MCCV), dependent on presence of risk factors**

5% (greater protection in most instances) after potential confounding risk factors had been taken into account (table 2). Multivariate analysis was not possible because of the small sample size.

We also estimated the protective effectiveness of the vaccine by the screening method, using population vaccination coverage for the different age groups.[1] This analysis gave an estimated protective effectiveness of 94% (73–98; p=0·015), which compares well with a previous estimated figure in teenagers (97%, 77–99).[1] Although narrower CIs for the protective effectiveness of the vaccine were noted with the screening method, this method is less reliable than the case-control approach, since it assumes absolute certainty of the proportion of vaccinees in the control group (by using population-based data). Should there be significant heterogeneity in vaccination coverage of the population, this method potentially introduces systematic error in the estimation of protective effectiveness.[4] The case-control method provides a more representative estimation of vaccine coverage for the population at risk.

We had little power to explore the effect of potentially confounding variables, since there were only 12 matched controls. However, comparison of these controls with the remaining risk factor study controls (and the cases) showed comparable proportions, suggesting that the estimate of efficacy would have changed little had more matched controls been recruited.

Identification of our cases through the Public Health Laboratory Service Meningococcal Reference Unit, Manchester, ensured accuracy of diagnosis, which reduced the chance of misclassification error (in terms of specificity) and increased the accuracy of the estimate of protective effectiveness.[5] Potential selection bias in identification of matched controls was diminished by restriction of control selection to the nearest in age on the general practitioner's list.

Our data provide further evidence that the meningococcal C conjugate vaccine is protective against invasive meningococcal C disease in teenagers, and indicate that the screening method used previously gave a reliable estimate of protective effectiveness.

Contributors

R Booy and R Viner contributed to study design, analysis, and writing of the report. A Bose and J Tully obtained data and drafted the manuscript. P Coen undertook statistical analysis and edited the report.

Conflict of interest statement

Prof R Booy has acted as a paid consultant for Wyeth.

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Low plasma arginine concentrations in children with cerebral malaria and decreased nitric oxide production

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Nitric oxide (NO) production and mononuclear cell NO synthase 2 (NOS2) expression are high in healthy Tanzanian children but low in those with cerebral malaria. Factors that downregulate NOS2 also diminish factors involved in cellular uptake and biosynthesis of L-arginine, the substrate for NO synthesis. We therefore postulated that L-arginine concentrations would be low in individuals with cerebral malaria. We measured concentrations of L-arginine in cryopreserved plasma samples from Tanzanian children with and without malaria. L-arginine concentrations were low in individuals with cerebral malaria (mean 46 μmol/L, SD 14), intermediate in those with uncomplicated malaria (70 μmol/L, 20), and within the normal range in healthy controls (122 μmol/L, 22; p<0·0001). Analysis by logistic regression showed that hypoargininaemia was significantly associated with cerebral malaria case-fatality. Hypoargininaemia may contribute to limited NO production in children with cerebral malaria and to severe disease.

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We have previously shown[1] an inverse association between falciparum malaria disease severity and both nitric oxide (NO) production and mononuclear cell NO synthase 2 (NOS2) expression, or both, with lowest levels noted in cerebral malaria. NO has antiparasitic effects in vitro and probably protects against disease by decreasing amounts of proinflammatory cytokines, reducing expression of endothelial cell surface adhesion molecules, and preventing

	Healthy controls (n=19)	Uncomplicated malaria (n=17)	Cerebral malaria (n=39)
Age (years)	5·3 (2·3)	3·6 (1·9)	3·5 (1·9)
Boys (numbers, %)	10 (53%)	10 (59%)	22 (56%)
Weight (kg)*	16·8 (5·0)	12·2 (3·0)	12·1 (3·3)
Time from last meal (h)	13 (1)	9 (12)	13 (11)
Time from last meal (range) (h)	11–16	0–48	1–48
White blood cell count (×10³/μL)	7·7 (1·8)	10·2 (4·4)	17·4 (10·9)
White blood cell count (range) (×10³/μL)	5–11·3	4·9–17·7	4·8–51·5
Haemoglobin (g/L)	111 (18)	77 (26)	64 (22)
Creatinine (μmol/L)	36·3 (6·7)	38·5 (8·8)	63·7 (38·2)
Parasitaemia (geometric mean) (trophozoites/μL)	321†	46 790	68 286

Values are mean (SD) unless otherwise indicated. *At enrolment before rehydration. †Four controls noted to have asymptomatic parasitaemia by microscopy of blood film.

Baseline characteristics

parasite cytoadherence. The reason why concentrations of NO are lower in patients with cerebral malaria than in healthy individuals is, however, unclear. NO is derived from L-arginine via NOS catalysis.[2] Cytokine responses can modulate NOS2 expression in coordination with proteins important for L-arginine uptake (cationic aminoacid transporters) and recycling from L-citrulline (argininosuccinate synthase).[2] Because NOS2 and L-arginine availability are both regulated by the host's cytokine response, we postulated that L-arginine concentrations would be reduced in individuals with cerebral malaria.

We measured concentrations of L-arginine in available cryopreserved plasma samples from three previously defined groups of Tanzanian children; healthy controls, and those with uncomplicated falciparum malaria and cerebral malaria.[1] From patients, blood samples were obtained at the time of diagnosis on admission to hospital. For controls, healthy individuals were fasted overnight and samples obtained the next day. Protocols were approved by the ethics committees at Muhimbili Medical Centre, Tanzania, University of Utah, USA, and Duke University, USA. Informed consent was obtained from all individuals at time of enrolment.[1]

From every sample, we passed 10 μL of plasma over an Oasis MCX cation exchange cartridge (Waters, Milford, MA, USA), according to the manufacturer's instructions. We dried out samples and resuspended them in 10 mmol/L sodium acetate, pH 4·5, before measuring concentrations of L-arginine with high performance liquid chromatography as previously described.[3] L-arginine and L-histidine eluted as discrete peaks and were quantified by use of standard curves. Intra-assay variability was 6·7%, and interassay variability was 8·3%.

Disease category means were compared with ANOVA with Bonferroni multiple comparison procedure. Linear regression and logistic regression were used to adjust for effects of potential confounding variables.

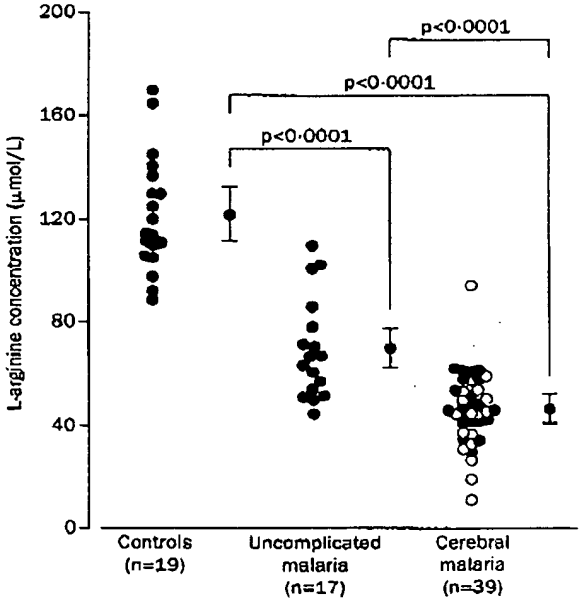
The table shows the baseline characteristics of individuals from whom samples were obtained. Samples were available from 19 controls, 17 patients with uncomplicated malaria, and 39 individuals with cerebral malaria (18 of whom died). Children with uncomplicated and cerebral malaria were younger, had lower mean haemoglobin concentrations and prehydration weights, and had higher white blood cell counts and *Plasmodium falciparum* parasitaemia than did controls (table).

L-arginine concentrations were inversely associated with disease severity (ANOVA p<0·0001). After Bonferroni adjustment for multiple comparisons, all disease-category means were significantly different from each other (figure). Mean plasma concentrations of L-arginine from 23 samples that we randomly collected from healthy American adults and from 11 cryopreserved samples from healthy, pregnant, Tanzanian women were 117 μmol/L (SEM 6) and 140 μmol/L (14), respectively. Thus, the values noted for control Tanzanian children are in line with those for other healthy control groups.

A regression model, controlling for enrolment characteristics, showed that disease severity was associated with L-arginine concentrations (p<0·0001). By contrast, mean L-histidine values did not differ significantly between groups (controls 157 μmol/L [SD 19]; uncomplicated malaria 101 μmol/L [42]; cerebral malaria 127 μmol/L [18]), with the use of a multivariate analysis controlling for the same variables (p=0·79).

To ascertain whether or not hypoargininaemia was associated with case-fatality, we used logistic regression to model the relation between L-arginine concentrations and survival in individuals with cerebral malaria, after adjustment for potential confounders (duration of fasting, Blantyre coma score, haemoglobin, white blood cell count, and parasitaemia). L-arginine concentration was analysed objectively as a dichotomous variable with the median amount in the group with cerebral malaria (45·4 μmol/L) as the breakpoint. In both univariate (odds ratio 4·0, 95% CI 1·1–15·2) and multivariate analyses (26·2, 1·2–586), L-arginine concentrations below the median were associated with death. With the same multivariable model, analysing L-arginine as a continuous variable, higher rather than lower L-arginine concentrations were protective against death (0·8, 0·71–0·98).

Plasma L-arginine concentrations were inversely associated with severity of malaria in Tanzanian children, and hypoargininaemia was associated with death in individuals with cerebral malaria. Whether or not severe disease is caused by, or results from, hypoargininaemia is unclear. However, patients with sickle cell disease and vaso-occlusive syndrome, and patients with lysuric protein intolerance (an inherited



Plasma L-arginine concentrations in healthy children, and in those with uncomplicated and cerebral malaria

Dot and bars=mean (SEM). In cerebral malaria, closed circles=recovery, and open circles=death.

disease of L-arginine deficiency due to mutations in cationic aminoacid transporters) have endothelial dysfunction that is related to hypoargininaemia and reduced NO production. L-arginine therapy restores NO production in sickle cell disease and lysuric protein intolerance,[3] and endothelial function in lysuric protein intolerance.[4] Endothelial dysfunction is important in cerebral malaria, a condition in which cytoadherence of parasite-infected red blood cells to endothelial cells is increased by cytokine-induced endothelial adhesion molecules. Intracellular NOS activity is dependent on adequate circulating concentrations of L-arginine. In our study, L-arginine concentrations in individuals with cerebral malaria were well below the transport velocity constant (K_m) for the cationic aminoacid transporter systems required for cellular L-arginine uptake. Thus, decreased expression of NOS2 coupled with plasma hypoargininaemia could act in concert to preclude optimum monocyte, endothelial, and tissue NO production in patients with cerebral malaria. Additionally, at low L-arginine concentrations, NOS enzymatically produces oxygen to superoxide without producing NO. Superoxide plus NO (assuming some is produced) react to form peroxynitrite. This reaction might enhance the deleterious oxidative stress noted in cerebral malaria.

Dietary factors and underlying malnutrition are unlikely to explain hypoargininaemia in individuals with cerebral malaria. Mean duration of fasting did not differ between groups and there was no independent association between weight-for-age and L-arginine. In those with cerebral malaria, weight-for-age was inversely associated with L-arginine concentrations. Amounts of glutamine and glutamate[5] and the essential aminoacid L-histidine are not reduced in severe malaria, further suggesting that our findings are not diet-related and are unlikely to be due to non-specific aminoacid catabolism in severe illness.

Alternative explanations for hypoargininaemia in cerebral malaria include inadequate enterocyte absorption, impaired endogenous biosynthesis or recycling, and depletion of L-arginine. Cellular uptake occurs through cationic aminoacid transporters with T helper 1-induced cationic aminoacid transporter 2 increasing uptake.[2] Enterocytes convert L-glutamine or glutamate to L-citrulline, which is converted to L-arginine in renal tubules. In blood monocytes, L-citrulline is recycled to L-arginine through argininosuccinate lyase and T helper 1-inducible argininosuccinate synthase. By contrast, T helper 2 cytokines increase monocyte arginase I and II, which catabolise L-arginine. We postulate that excessive T helper 2 responses in cerebral malaria (as evidenced by high interleukin 10 concentrations in these children[1]) decrease NOS2 expression, as well as L-arginine uptake and biosynthesis. Extrahepatic arginase activity might also be increased, further reducing L-arginine concentrations. Additional mechanistic studies are required to address these notions.

Irrespective of the cause, hypoargininaemia probably limits NO production and is associated with death in cerebral malaria. Strategies to increase NO production could improve outcome by correcting endothelial dysfunction and decreasing deleterious inflammatory cytokine responses. Clinical trials are warranted to ascertain whether or not correction of L-arginine deficiency provides adjunctive prophylactic and therapeutic benefit in malaria.

Contributors

B K Lopansri, N M Anstey, J B Weinberg, and D L Granger designed the study. N M Anstey and E D Mwaikambo enrolled participants and obtained samples. Development of assays and biochemical analysis was done by B K Lopansri and D L Granger. B K Lopansri, N M Anstey, and G J Stoddard collected and analysed data. N M Anstey, J B Weinberg, and D L Granger supervised the study. All authors contributed to the interpretation of results and writing of the report.

Conflict of interest statement

None declared.

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Umbilical-cord blood for transfusion in children with severe anaemia in under-resourced countries

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Shortage of blood for transfusion contributes substantially to mortality of children with severe anaemia in sub-Saharan Africa. Umbilical-cord blood could be an additional and readily available source of blood. We aimed to show whether it is possible to gather cord blood in a busy Ghanaian labour ward. Mean volume of each blood sample obtained from the umbilical cord was 85 mL (SD 28·0). This amount of blood is sufficient to raise the haemoglobin concentrations of 28 (21%) of 131 children needing transfusions in the same hospital, by 30 g/L. Further work is needed to improve the sterility of cord blood and to establish the resource and logistical implications of scaling-up for sub-Saharan Africa transfusion services.

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Severe anaemia in children is a major health problem in sub-Saharan Africa. In most children, severe anaemia is associated with *Plasmodium falciparum* infection, and those younger than 2 years old are most frequently affected. In countries where malaria is endemic, up to 45% of all hospitalised children have a haemoglobin concentration of less than 50 g/L, and in-hospital death rates of patients are 8–18%.

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THE CLINICAL PHARMACOLOGY OF L-ARGININE

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Key Words endothelium, nitric oxide, cardiovascular disease, pharmacokinetics, side effects

■ **Abstract** L-Arginine (2-amino-5-guanidinovaleric acid) is the precursor of nitric oxide, an endogenous messenger molecule involved in a variety of endothelium-mediated physiological effects in the vascular system. Acute and chronic administration of L-arginine has been shown to improve endothelial function in animal models of hypercholesterolemia and atherosclerosis. L-Arginine also improves endothelium-dependent vasodilation in humans with hypercholesterolemia and atherosclerosis. The responsiveness to L-arginine depends on the specific cardiovascular disease studied, the vessel segment, and morphology of the artery. The pharmacokinetics of L-arginine have recently been investigated. Side effects are rare and mostly mild and dose dependent. The mechanism of action of L-arginine may involve nitric oxide synthase substrate provision, especially in patients with elevated levels of the endogenous NO synthase inhibitor asymmetric dimethylarginine. Endocrine effects and unspecific reactions may contribute to L-arginine-induced vasodilation after higher doses. Several long-term studies have been performed that show that chronic oral administration of L-arginine or intermittent infusion therapy with L-arginine can improve clinical symptoms of cardiovascular disease in man.

BIOCHEMISTRY AND PHYSIOLOGICAL ROLES OF L-ARGININE

L-Arginine (2-amino-5-guanidinovaleric acid) is a basic, semiessential amino acid. Its occurrence in mammalian protein was discovered by Hedin in 1895 (1). At that time, the existence of L-arginine as a naturally occurring molecule had already been known since 1886, when it was first isolated from lupin seedlings (2). Our present knowledge about the involvement of L-arginine in several different metabolic pathways is the result of discoveries that were made during the 20th century (Figure 1). One was that synthesis of L-arginine and its subsequent disintegration into L-ornithine and urea, catalyzed by the activity of arginase, is a

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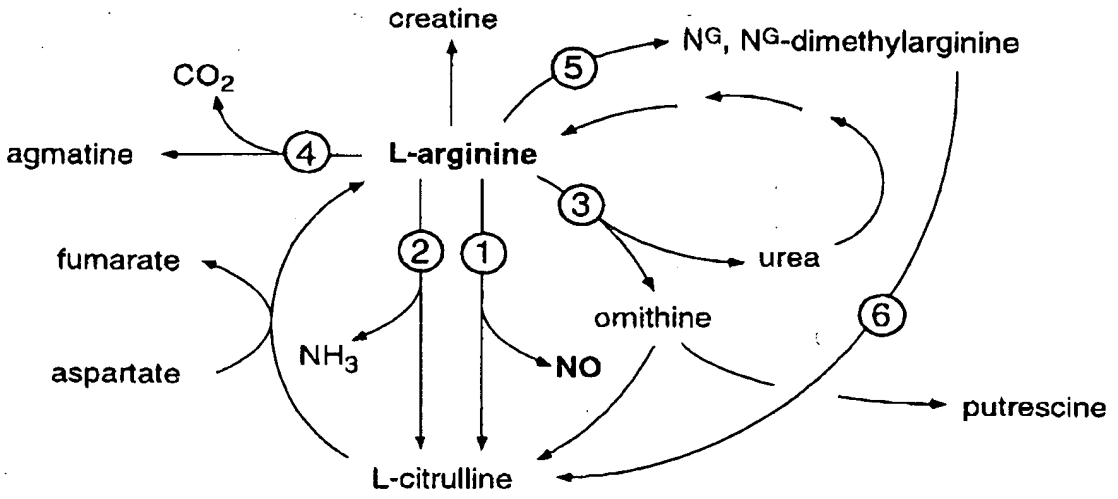

Figure 1 Schematic representation of the metabolism of L-arginine. Enzymatic pathways indicated by numbers: 1, biosynthesis of nitric oxide (NO) from L-arginine by nitric oxide synthase; 2, synthesis of L-citrulline from L-arginine by arginine deaminase; 3, conversion of L-arginine to urea and L-ornithine by arginase (part of the urea cycle); 4, decarboxylation of L-arginine to agmatine by arginine decarboxylase; 5, methylation of L-arginine by protein arginine *N*-methyltransferases; 6, metabolism of dimethylarginine to L-citrulline by dimethylarginine dimethylaminohydrolase.

ubiquitous pathway that serves to eliminate nonessential nitrogen-containing substances from the body. In 1932, Krebs & Henseleit reported their finding that L-arginine is an essential component in this cyclic metabolic pathway, the urea cycle (3). This is the only pathway in mammals that allows elimination of continuously generated toxic ammonia from the body. Furthermore, the "byproduct" of this reaction, L-ornithine, is a precursor for synthesis of polyamines, molecules essential for cell proliferation and differentiation (4). In 1939, Foster et al (5) discovered that L-arginine is also required for the synthesis of creatine. In its phosphorylated form (creatine phosphate), creatine is an essential energy source for muscle contraction. Its degradation product, creatinine, is eliminated by glomerular filtration in the kidney and is used as a surrogate measure of glomerular filtration rate. In the 1980s it was discovered that L-arginine is a precursor of nitric oxide (NO) (6–8), the chemical entity shown to be identical with endothelium-derived relaxing factor (9). The enzyme that synthesizes NO from the terminal guanidino nitrogen group of L-arginine was isolated, cloned, and characterized in 1991 from macrophages (10), endothelial cells (11), and neuronal cells (12). In the 1990s, the enzyme arginine decarboxylase was discovered in mammalian cells (13). This enzyme converts L-arginine into agmatine, a molecule whose physiological functions are still under investigation. Agmatine has been shown to bind to α_2-adrenoceptors and imidazoline receptors, potentially evoking clonidinelike effects on blood pressure (13). Agmatine is also a weak inhibitor of NO synthase (NOS) isoenzymes, suggesting a possible role for it as an endogenous modulator of NO production if local concentrations are sufficiently high (14).

In early studies L-arginine was characterized as a nonessential amino acid in the healthy adult human (15) but as an essential amino acid for young, growing animals (16). Homeostasis of plasma L-arginine concentrations is regulated by dietary arginine intake, protein turnover, arginine synthesis, and metabolism. This may explain why, under certain disease conditions, L-arginine may become an essential dietary component. The main tissue in which endogenous L-arginine synthesis occurs is the kidney, where L-arginine is formed from L-citrulline, which is released mainly by the small intestine (17). The liver is also capable of synthesizing considerable amounts of L-arginine; however, this is completely reutilized in the urea cycle so that the liver contributes little or not at all to plasma L-arginine flux (18). The nearly complete separation between hepatic and systemic L-arginine pools has also been partly attributed to the fact that the active L-arginine uptake system, the y^+ transporter (19), has a very low activity in hepatocytes (20). Cell types containing NOS have been demonstrated to be able to reutilize L-citrulline, the byproduct of NO synthesis, to L-arginine via the so-called arginine-citrulline cycle (21, 22). This pathway is mediated by enzymes also involved in the hepatic urea cycle; however, the fact that L-citrulline accumulates in the medium of NO-producing cells indicates that the arginine-citrulline cycle is far less efficient than the urea cycle (23). In macrophages and other cell types, induction of inducible NOS is accompanied by induction of argininosuccinate synthase, the rate-limiting enzyme of L-arginine biosynthesis (24), suggesting that NOS substrate availability is tightly regulated and may be rate limiting for NO production under certain conditions.

Approximately 5.4 g of L-arginine is absorbed each day in adults who ingest an average US diet (25). Thus, each gram of dietary protein supplies about 54 mg of L-arginine. Walser (26) estimated that a 70-kg adult person eating about 50 g of protein per day consumes about 0.2 mmol (34.8 mg) of L-arginine per kg of body weight per day or a total of 2.4 g of L-arginine per day. The difference between the two studies is because of differences in the estimates of average protein intake in usual Western diets. Average L-arginine intake can therefore be assumed to be on the order of 4–5 g/day.

ROLE OF L-ARGININE AS A PRECURSOR OF NITRIC OXIDE: Preclinical Pharmacology

L-arginine is the precursor for the endogenous synthesis of NO due to the activity of NOS, which releases L-citrulline as a byproduct (6–8). Although only a minor portion of L-arginine is metabolized via this pathway in vivo, it has attracted much interest in recent years because of the prominent role that NO plays in vascular physiology and pathophysiology (for reviews, cf 27, 28). NO generated from L-arginine is a highly reactive radical gas and an important messenger molecule that is involved in functions as diverse as neurotransmission, vasodilation, inflammation, and regulation of gene expression. At low concentrations like those produced by constitutive endothelial NOS (ecNOS) in the vasculature in vivo,

NO acts as a paracrine-signaling molecule, mediating vasodilation (29), inhibition of platelet activation (30), inhibition of monocyte and leukocyte adhesion (31), and inhibition of smooth muscle cell proliferation (32) and controlling vascular oxidative stress and the expression of redox-regulated genes (33).

In certain animal models and in human diseases (see below), the biological functions of endothelium-derived NO are impaired, leading to dysregulation of endothelial control of vascular tone and blood flow. Such models include hypercholesterolemic rabbits (34–36), rat models of hypertension (37), and hyperlipidemic monkeys (38). The mechanisms behind this phenomenon are probably multifactorial, including reduced NO elaboration by NOS, increased oxidative inactivation of NO, and enhanced formation of vasoconstrictor mediators like endothelin-1 and thromboxane A_2 (28, 39).

What is the role of L-arginine in this setting? NOS is inhibited by L-arginine analogs that are substituted at the guanidino nitrogen atom, like N^G-monomethyl-L-arginine or N^G-nitro-L-arginine (40). Inhibitory action of these molecules is overcome by excess L-arginine (40), indicating that there is competition for enzyme binding between L-arginine and its inhibitory analogs. Reduced activity of endothelial cell NOS was also shown to occur in the presence of low-density-lipoprotein cholesterol; again, this effect can be overcome by excess L-arginine (41). Although the mechanism behind this latter phenomenon has not yet been fully elucidated, these data demonstrate that, under certain conditions, L-arginine availability regulates endothelial cell NOS activity.

On the other hand, depletion of L-arginine in endothelial cells is hardly possible, due to high intracellular L-arginine concentrations (42) and the ability of endothelial cells to synthesize L-arginine from L-citrulline (21). Incubation of isolated blood vessels with high concentrations of L-arginine does not directly affect vascular tone, nor does it modulate endothelium-dependent relaxation (43). However, a plethora of reports from animal studies have shown that acute or chronic administration of L-arginine in vivo improves vascular responsiveness, probably via enhanced NO elaboration. Acute administration of L-arginine augments endothelium-dependent vasodilation in cholesterol-fed rabbits (34, 44) and transiently increases urinary excretion of nitrate (the metabolite of NO) in rats (45). Apart from these acute effects, long-term oral administration of L-arginine has been associated with a significant improvement in NO-dependent vasodilation in cholesterol-fed rabbits (35, 36, 46, 47) and in low-density-lipoprotein receptor knockout mice (48). In these animal models, other NO-dependent vascular functions are also modulated by chronic supplementation with L-arginine: Endothelial leukocyte adhesion is reduced (49), platelet aggregation is inhibited (50, 51), and vascular smooth muscle cell proliferation in vivo is attenuated (52). The latter effect may prominently contribute to reduced restenosis after experimental angioplasty (53–55) and to reduced intimal thickening in vein grafts (56).

L-arginine treatment influences the progression of the atherosclerotic disease process in animal models: When administered via the oral route to rabbits fed a cholesterol-enriched diet, development of intimal plaques in the carotid arteries

is slowed (35, 36), the intima/media ratio in the thoracic and abdominal aorta is reduced (35, 36, 46), and intimal thickening in the coronary arteries is inhibited (57). Enhanced NO elaboration after L-arginine supplementation also contributes to improved perfusion of collaterals after arterial occlusion in rabbit ear tissue (58). There is controversy about whether L-arginine can induce regression of preexisting lesions. Candipan and coworkers (47) demonstrated that, after 10 weeks on a high-cholesterol diet, supplementation with L-arginine for an additional 13 weeks led to significant reduction in aortic lesion size. We (36) found that addition of L-arginine to the diet for 8 weeks after 4 weeks of preceding hypercholesterolemia completely halted the progression of vascular lesions in the aorta and carotid artery of rabbits, but did not induce regression. Inhibition of smooth-muscle-cell proliferation by L-arginine (52) and induction of apoptotic cell death in vascular lesions (59) may both contribute to the beneficial effects of L-arginine on vascular structure in this animal model.

FROM BENCH TO BEDSIDE: Cardiovascular Effects of L-Arginine in Humans

Very soon after the first animal experiments had proven a beneficial effect of L-arginine on endothelial function, it was shown that local intracoronary infusion of L-arginine normalized coronary vasomotor responses to acetylcholine in hypercholesterolemic humans (60). A similar observation was also made upon systemic (intravenous) infusion of L-arginine in hypercholesterolemic subjects, in whom endothelium-dependent forearm vasodilation was improved (61). These were important findings, because endothelial dysfunction precedes angiographically visible atherosclerotic lesions in large coronary arteries (62). Recent evidence from prospective clinical trials suggests that endothelial dysfunction is a predictor of future coronary events (63, 64). Therefore, reversal of endothelial dysfunction by L-arginine in vivo may suggest that this amino acid exerts antiatherosclerotic effects in humans.

The responsiveness of endothelial dysfunction to L-arginine is not a ubiquitous phenomenon. It depends on factors such as the arterial segment studied, the presence or absence of morphological atherosclerotic lesions, the underlying cardiovascular disease, and the L-arginine concentration reached. Egashira et al (65) showed that coronary vasodilator response to acetylcholine was significantly improved after intracoronary L-arginine in patients with microvascular angina and normal coronary angiograms. Drexler et al (66) demonstrated that, in cardiac transplant recipients, improvement of coronary endothelial function with L-arginine is more likely in vessels with normal wall morphology. By contrast, in another study a more prominent vasodilator effect of L-arginine was found in stenosed coronary arteries than in healthy vessel segments (67).

Although there is a bulk of evidence that supplementation with L-arginine—via the intraarterial, intravenous, or oral route—improves endothelial dysfunction in

hypercholesterolemia and atherosclerosis, endothelial dysfunction in other cardiovascular diseases was not consistently improved by L-arginine administration. The majority of studies with L-arginine in hypertensive patients revealed a lack of effect of this amino acid on endothelial function (68, 69). In a comparative trial between patients with coronary artery disease and patients with primary pulmonary hypertension, we also found no vasodilator effect of L-arginine in pulmonary hypertension (70). Reduced NO elaboration in certain types of hypertension may be caused by reduced expression of endothelial NOS (71). This may explain why increased substrate availability has no beneficial effect in this condition.

In a series of clinical studies, we investigated direct hemodynamic effects of L-arginine in the peripheral vasculature. We found that intravenous infusion of 30 g of L-arginine significantly increased arterial blood flow in the femoral artery of healthy subjects by a mean 44% (72). In a subsequent study, the peripheral vasodilator action of 30 g of L-arginine was reproduced by using impedance cardiography to assess total peripheral resistance (73). Plasma L-arginine concentrations increased to 6.0 ± 0.4 mM. A lower dose of L-arginine (6 g), administered by either the intravenous or the oral route, failed to produce acute vasodilation. Plasma L-arginine concentrations rose to 822 ± 59 μM and 310 ± 152 μM after intravenous and oral administration, respectively, of 6 g of L-arginine.

In a study in patients suffering from severe peripheral arterial occlusive disease, we demonstrated that an acute intravenous infusion of 30 g of L-arginine increased femoral arterial blood flow in the more severely affected leg by a mean 43% (74). In this study the plasma L-arginine concentration increased to a mean 3.8 ± 0.4 mM. This vasodilator effect of L-arginine, which was measured by duplex ultrasonography in the femoral artery, was due to increased blood flow velocity, whereas femoral artery diameter remained unchanged. Although this observation pointed to a peripheral vasodilator action of L-arginine, we had no evidence from that study about whether that action involved opening of arteriovenous shunts (which, in the long run, might further decrease nutritive muscle blood flow in the diseased limb) or increasing muscle capillary blood flow (which might prove to be therapeutically favorable for peripheral arterial occlusive disease patients). We addressed this question in a further clinical study in which we performed serial measurements of muscle capillary blood flow by using positron emission tomography with isotope-labeled water as the flow tracer. We found that a single systemic infusion of 30 g of L-arginine increased nutritive muscle blood flow by a mean 43%, whereas a lower dose of 8 g of L-arginine had no significant effect (75).

Increased nutritive tissue blood flow, as well as our observation that blood flow remained elevated for 1–2 h after the end of L-arginine infusion (72, 74), made us confident that, on a medium- to long-term scale, L-arginine might improve the symptoms of occlusive arterial disease. In the first study to address this question, we found that, after 3 weeks of intermittent intravenous L-arginine therapy (3 doses of 8 g/day), claudication distance was significantly increased (76). Absolute and pain-free walking distances were improved by 230 ± 63% and 155 ± 48%,

respectively, whereas in the control group there were no significant changes. Six weeks after the end of the infusion therapy, walking distance was still significantly prolonged, which may be a result of persistent improvement of endothelium-dependent vasodilation in response to increased peripheral blood flow (e.g. during walking exercise).

Other investigators also showed that oral L-arginine supplementation improves clinical symptoms of vascular disease (see Table 1, p. 91): Ceremuzynski et al (77) showed that exercise capacity was improved as compared to placebo in patients with stable angina pectoris after 3 days of 6 g of L-arginine daily. Six months of oral treatment with L-arginine (3 doses of 3 g/day) resulted in a significantly improved angina symptom score and improved coronary blood flow response to acetylcholine in another placebo-controlled study that included 26 patients with small-vessel coronary artery disease (78). By contrast, Blum et al (79) observed no improvement of NO elaboration, flow-mediated vasodilation of the brachial artery, or serum adhesion molecule levels after 1 month of oral L-arginine (9 g/d) in patients with coronary artery disease. However, in that study, patients were on an optimized medical treatment including cholesterol-lowering and vasoactive medication before and during the study, and flow-mediated vasodilation was normal at baseline, which may have left too little room for improvement by L-arginine.

Acute intravenous infusion of 20 g of L-arginine resulted in significantly reduced peripheral resistance, increased stroke volume, and cardiac output without a change in heart rate in 12 patients with congestive heart failure (80). In a placebo-controlled study with oral L-arginine (3.6–12.6 g/d over 6 weeks), Rector et al (81) found significant improvements in forearm blood flow, increased walking distance in a 6-min walk test, and improved arterial compliance and quality of life in patients with heart failure.

In patients with Raynaud's phenomenon and scleroderma, vasospastic attacks are significantly reduced by L-arginine, suggesting that NO deficiency may be involved in the pathogenesis of vasospasms in Raynaud's phenomenon (82).

MECHANISM(S) OF ACTION OF L-ARGININE'S CARDIOVASCULAR EFFECTS IN HUMANS

The first assumption of the mechanism behind the vascular effects of L-arginine in vivo was that it acts via substrate provision for NOS (34). However, this assumption was controversial because of the obvious discrepancy between the half-saturating L-arginine concentration (K_m value) for isolated, purified endothelial NOS [2.9 μM (11)] and plasma L-arginine concentration (60–100 μM). From an enzyme-biochemical point of view, it was argued that additional L-arginine could not have any effect on NOS activity, because this enzyme should be saturated with substrate at physiological levels and not be dependent on extracellular supply. However, L-arginine did have a beneficial effect on endothelium-dependent vasodilation in vivo. This phenomenon was called the "L-arginine paradox." Several

explanations have been brought forward to resolve this paradox. First, L-arginine may be compartmentalized in the cytoplasm, and local concentrations in the vicinity of endothelial NOS may be lower than expected from L-arginine levels in whole-cell homogenates. Indeed, there is recent evidence that endothelial NOS is colocalized in caveolae formed by the cytoplasmic membrane with the y^+ transporter (83). Extracellular L-arginine may be preferentially utilized by NOS within this microenvironment. This may explain earlier findings showing rapid conversion of extracellular L-[guanidino-$^{15}N_2$]-arginine into ^{15}N-nitrate by cultured endothelial cells (8).

Another explanation for the L-arginine paradox may be the presence of endogenous NOS inhibitors in certain diseases. Presence of asymmetric dimethylarginine (ADMA), an endogenous molecule that exerts NOS-inhibitory effects, has been demonstrated in human plasma and urine (84). Elevated concentrations of ADMA are present in patients with vascular diseases, resulting in diminished NOS activity (see below). As discussed above for synthetic L-arginine analogs, inhibition of NOS activity may be overcome by excess substrate and could explain how L-arginine improves endothelial function in patients with vascular disease. However, this mechanism would not explain L-arginine-induced vasodilation in healthy humans in whom ADMA levels are low.

Endocrine mechanisms may contribute to vasodilation induced by L-arginine in healthy humans and in patients. High intravenous doses of L-arginine (30 g) have been used since the 1960s to stimulate growth hormone (GH) secretion (85). In addition, L-arginine stimulates the release of pancreatic insulin (86) and glucagon (87) and pituitary prolactin (88). Of these, GH and insulin can induce vasodilation by mechanisms that have long remained unclear. Giugliano and coworkers (89) recently demonstrated that an intravenous infusion of 30 g of L-arginine induced vasodilation and insulin release in healthy humans. When insulin secretion was blocked by octreotide coinfusion, no vasodilation occurred. However, vasodilation was restored by insulin coadministration. Unfortunately, these investigators did not measure GH release, which is also blocked by octreotide. We were able to show that L-arginine (30 g, intravenously) induced a rapid release of insulin and a delayed release of GH (90). During coinfusion of somatostatin, release of both hormones was blocked; however, somatostatin inhibited only the late response but not the early increase in NO production. Our conclusion therefore was that GH contributes to the prolonged NO-dependent vasodilation to high doses of L-arginine. Other studies corroborate this hypothesis. GH exerts many of its effects via insulinlike growth factor-1 (91). Insulinlike growth factor-1 activates ecNOS in vitro (92, 93), and it induces NO-dependent vasodilation in human forearm tissue in vivo (94). We have further shown that chronic administration of recombinant GH increases NO production in GH-deficient patients (95) and in patients with dilated cardiomyopathy (96). Oral administration of L-arginine in combination with L-lysine has also been found to stimulate GH release, but L-arginine alone given by the oral route has no such effect (97). Therefore, whether this mechanism contributes significantly to the beneficial effect of oral L-arginine

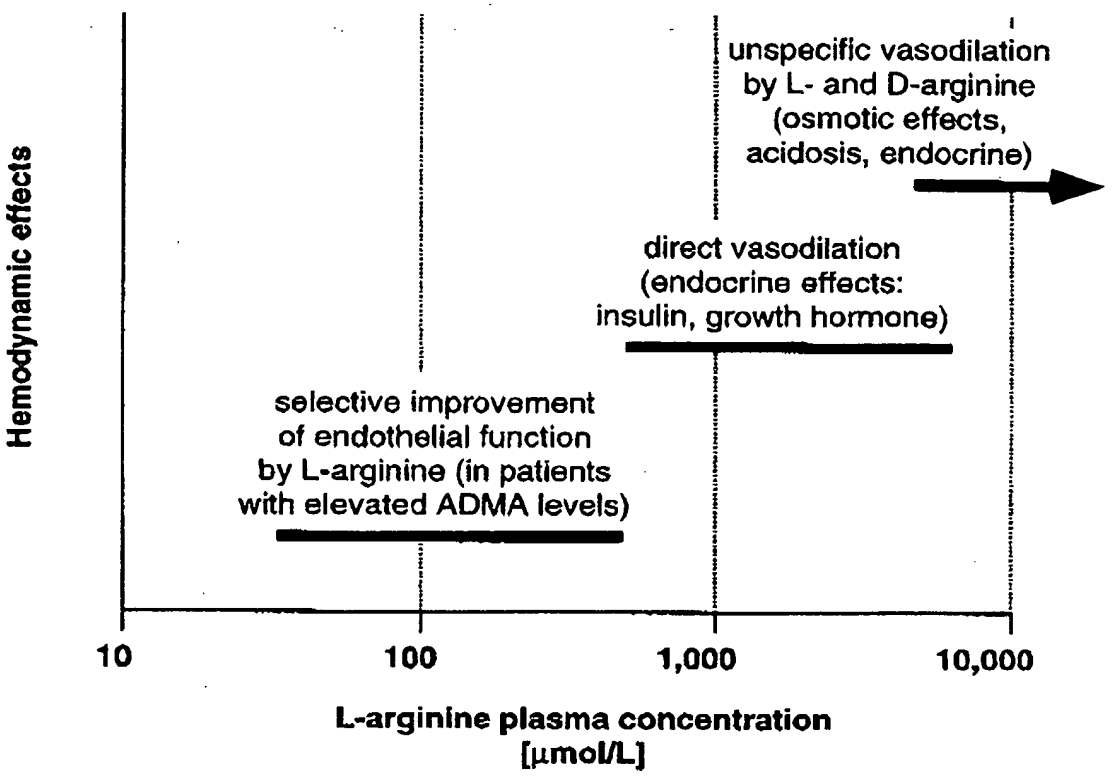

Figure 2 Concentration-dependent clinical effects of L-arginine as observed in studies in human subjects.

on endothelium-dependent vasodilation in humans is not known. We did not find evidence for increased GH secretion during oral L-arginine supplementation (2 doses of 8 g/day) in human subjects (S Bode-Böger & RH Böger, unpublished observation) (Figure 2).

Agmatine is another metabolite that may be involved in the vasodilatory action of L-arginine. Agmatine is a ligand at central α_2-and imidazoline receptors (13), where it induces clonidine-like effects (97) and—by lowering peripheral sympathetic tone—may lower blood pressure and induce vasodilation. No data are available to date showing whether this mechanism contributes to L-arginine-induced vasodilation in humans in vivo.

Unspecific effects have been suggested to be involved in vasodilation induced by high intravenous doses of L-arginine. MacAllister et al (88) reported that, after intravenous infusion resulting in concentrations in the millimolar range, neither L-arginine nor D-arginine had an effect on systemic hemodynamics in healthy humans or in patients with insulin-dependent diabetes or hypertension. Increases in plasma insulin, prolactin, glucagon, and growth hormone were seen with both enantiomers. However, as outlined above, the vasodilatory effect of GH is mediated by NO, which may explain why, in some experimental settings, D-arginine may have effects on endothelium-dependent vasodilation similar to the effects of L-arginine.

Antioxidant effects have been reported for L-arginine that may contribute to increased biophase availability of NO. We found that superoxide radical release by isolated aortic rings from cholesterol-fed rabbits was significantly diminished after L-arginine supplementation (35). We later confirmed our findings using a different approach, that is, by showing reduced urinary excretion of 8-iso-prostaglandin $F_{2\alpha}$, a marker for lipid peroxidation in vivo (99). Reduced superoxide elaboration by endothelial cells has been shown to be specific for L-arginine but not D-arginine (100), suggesting that it may be related to NOS activity. Indeed, NOS catalytic activity may be decoupled under certain pathophysiologic conditions, resulting in a shift of catalytic activity from NO elaboration to O_2^- production (101); this effect can be reversed by L-arginine (102). In vivo, L-arginine treatment decreases NOS-derived superoxide while increasing NO accumulation during ischemia/reperfusion injury in skeletal muscle (103). We have evidence from our laboratory showing that isoprostane excretion is reduced in humans with vascular disease during L-arginine treatment (RH Böger & D Tsikas, unpublished observation).

SIDE EFFECTS

L-arginine has generally been well tolerated when administered via the intraarterial, intravenous, or oral route, in doses $\leq$30 g. When high doses of L-arginine are given intravenously, local irritation and phlebitis may occur due to the high osmolality of the solution (104, 105). Dilution to a 10% solution is recommended but not always feasible because high doses may cause fluid overload. Accidental extravasation of L-arginine solutions may lead to local tissue injury and necrosis (106). The vasodilator action of L-arginine may lead to hypotension (107), but usually, the blood pressure-lowering effect of L-arginine is relatively limited. Allergic reactions including anaphylaxis are possible in response to L-arginine (108). It is therefore contraindicated in individuals with high allergic tendencies. Antihistamines and epinephrine should be available for treatment of such reactions. Infusion solutions of L-arginine hydrochloride have a high chloride content; this may be hazardous in patients with electrolyte imbalance. Because the L-arginine hydrochloride solution is acidic (pH 5–6.5), a sudden drop in blood pH may cause metabolic acidosis, which has been associated with arrhythmias in a variety of clinical settings (109). Hyperkalemia, including one fatality, has been reported after L-arginine infusion in patients with severe liver disease and/or renal insufficiency (110–113). Massara et al (114) reported hyperkalemia and hypophosphatemia in diabetic patients receiving L-arginine infusions. It was suggested that hyperkalemia is the result of displacement of intracellular potassium by L-arginine, a cationic amino acid (115). Potassium clearance from plasma is enhanced by the action of insulin stimulated by L-arginine. In diabetics, lack of insulin may cause hyperkalemia. Stimulation of insulin release may cause hypoglycemia in patients with intact pancreatic function. Release of histamine from skin (116) may be a cause of flushing and other dermal side effects (117). A major part of an L-arginine

dose is metabolized to ornithine and urea. Increases in blood nitrogen urea and urea may occur in patients with renal function impairment owing to their limited capacity to eliminate urea. Oral L-arginine may cause nausea and vomiting; the frequency was given as ~3% of patients (118). Abdominal cramps and bloating have been observed in patients with cystic fibrosis receiving oral L-arginine (119). One fatal case due to an acute overdose of L-arginine was recently reported (120). A 21-month-old girl inadvertently received eightfold the dose of L-arginine that is routinely given to stimulate growth hormone release, and she died from cardiac arrest and myelinolysis. Patients receiving L-arginine infusions should be monitored closely for cardiac arrhythmias and electrolyte disturbances.

CLINICAL PHARMACOKINETICS OF L-ARGININE

There are only a few studies that have specifically addressed the pharmacokinetics of L-arginine in humans. In two older studies, arginine levels were measured by photometric methods, and owing to various limitations related to the route of administration, low assay sensitivity, short follow-up (15 and 60 min after the end of a 30-min infusion period), and data analysis, pharmacokinetic parameters obtained were unreliable (121, 122). In another study, Matera et al (123) tested the relative bioavailability and bioequivalence of two oral poly-amino acid formulations in association with vitamin B_{12} used as parenteral nutrition supplements. The dosage of L-arginine in these formulations was only 100 mg per day, and no data are available from that study on absolute bioavailability of L-arginine. More recently, Bode-Böger et al (72) compared the pharmacokinetics of single intravenous doses of L-arginine (30 and 6 g) with that of oral L-arginine (6 g), and Tangphao et al (124) studied the pharmacokinetics after administration of 30 g of L-arginine via the intravenous route and after oral administration of 10 g of L-arginine. The latter two studies, in combination with data from animal studies and metabolism studies, provide much of the pharmacokinetic data on L-arginine that are known to date.

After an intravenous infusion, peak plasma L-arginine levels are achieved within 20–30 min. Peak plasma levels range from 0.8 mM after 6 g of L-arginine (72) to 4.8 mM after 14 g of L-arginine (125) and to 6.2 mM (72), 6.5 mM (90), and 8.0 mM (124) after 30 g of L-arginine. The peak L-arginine plasma concentration after oral administration of 6 g is 0.31 mM at T_{max} = 90 min (72). Oral administration of 10 g of L-arginine leads to a peak plasma concentration of 0.29 mM at 60 min after dosing (124). These data suggest a less than proportional increase in plasma L-arginine after high doses, and indeed there is substantial urinary excretion of L-arginine when the renal threshold for reabsorption is exceeded (124).

Orally administered L-arginine is rapidly and almost completely absorbed by the intestinal brush border membrane via active uptake by the intestinal y^+ transporter system for cationic amino acids (19); thereafter, it is extensively metabolized by enterocytes (126). Data given for oral bioavailability vary between 21 ± 4% (5–50%) (124) and 68 ± 9% (51–87%) (72). Splanchnic uptake of isotope-labeled

L-arginine after oral administration was 31–38% in another study (127). Although the reason for these differences is not clear, it is obvious that a considerable fraction of an oral L-arginine dose is being metabolized presystemically or excreted from the gut. The liver may not contribute significantly to this first-pass metabolism, because the y^+ transporter in hepatocytes has a very low activity, almost completely separating hepatic arginine metabolism from the systemic L-arginine pool (128).

The half life of L-arginine was 1.5–2 h after an oral dose of 6 g (72). This value corresponds to the half life determined in an earlier study for two different poly-amino acid formulations, in which half life of L-arginine was reported to be 1.2–1.9 h (123). In the latter study, no change in L-arginine's half life was found after repeated intake. After higher intravenous doses of L-arginine, its apparent half life is shorter, which may be caused by a "spillover" into urine from the extremely high plasma concentrations reached (124).

Frondoza et al (129) assessed tissue distribution of [^{14}C]L-arginine after intraperitoneal injection of the tracer in rats and found the largest portions of radioactive label in the skin, liver, small intestine, and stomach. The disappearance of radioactivity from tissues gave semilog decay curves, suggesting that there are several labeled products with different turnover rates (129). The bulk of radioarginine was eliminated through the urine, probably after conversion of arginine to urea. In the same study, a similar observation was also made in humans with multiple myeloma, in whom 22–26% of injected [^{14}C]L-arginine was found in urine during the first 24 h. Cumulative radioactivity excreted until 8 days after injection was 70% of injected dose (129). In another study it was found that L-arginine levels increase to three- to sixfold of baseline levels in NO-generating tissues like the heart and aorta of rats (130). Beaumier et al (131) reported that the rate of conversion of arginine to ornithine significantly increased in humans with a high dietary L-arginine supplementation (36 g/day), with no apparent change in total body conversion of L-arginine to NO_3^-. Only a very small fraction of exogenous L-arginine is converted via NO into nitrate. Leaf et al (132) estimated this fraction as 0.07% of an intravenous dose of ^{15}N-labeled L-arginine; Castillo et al (133) calculated that 0.4% of ^{15}N-labeled L-arginine applied via the intragastric route was converted into ^{15}N-NO_3^-. These findings are in line with our recent observation that <1% of oral L-[guanidino-$^{15}N_2$]-arginine is converted into ^{15}N-NO_3^- in rabbits (RH Böger, S Bode-Böger, & D Tsikas, unpublished observation).

Under physiological conditions, excretion via the kidneys plays no role for the elimination of L-arginine. L-arginine is filtered in the renal glomerulus and almost completely [>99% (134)] reabsorbed in the proximal renal tubules (135) and in the thin ascending limb of Henle's loop (136). Reabsorption is accomplished by the y^+ transporter and—in contrast to other amino acids—displays saturation kinetics. In dogs, the renal tubular transport maximum for L-arginine is reached at a glomerular filtration of 3.5–4 mg/min/100 ml of glomerular filtrate (137). No such data are available for humans. However, the finding of L-arginine spillover into urine in human subjects after high intravenous doses of the amino acid (124) is consistent with saturable tubular reabsorption.

TABLE 1 Diseases in which L-arginine has been demonstrated to improve clinical end points of cardiovascular disease

Disease	L-arginine dose[a]	Effect[b]	Reference
Peripheral arterial disease	3 × 8 g/d i.v.	↑ Walking distance	76
	30 g i.v.	↑ Nutritive muscle blood flow	75
Coronary artery disease	3 × 3 g/d p.o.	↓ Angina symptom score	78
	3 × 2 g/d p.o.	↑ Exercise capacity	77
Congestive heart failure	5.6–12.6 g/d p.o.	↑ Exercise capacity	81
Raynaud syndrome	8.5 mg/min i.a.	↓ Vasospasm attacks	82

[a]Routes of administration: i.v., intravenously; p.o., orally; i.a., intra-arterially.

[b]↑, Increased; ↓, decreased.

SOLVING THE "L-ARGININE PARADOX"?

While many of the effects of high intravenous doses of L-arginine observed in clinical trials can be explained by endocrine actions of this amino acid, the improvement of endothelial function brought about by relatively low daily oral doses of L-arginine has long remained unclear. The in vivo actions of L-arginine were in contrast to the absence of similar effects in vitro and were not predictable based on the huge excess of physiological L-arginine concentrations over the apparent K_m value of NOS isoenzymes as determined in vitro. The presence of an endogenous inhibitor of NOS may account for L-arginine's effects in many cardiovascular and other diseases. The endogenous NOS inhibitors, N^{ω}-monomethyl-L-arginine and ADMA have been detected in human plasma and urine (84); ADMA is present in concentrations $\leq$10-fold higher than those of N^{ω}-monomethyl-L-arginine. Elevated concentrations of ADMA have been found in patients with peripheral arterial occlusive disease (138), hypercholesterolemia (125), chronic heart failure (139), end-stage renal disease (140), hyperhomocysteinemia (141), and hypertension (142). Elevated ADMA levels account for endothelial dysfunction in hypercholesterolemia and hyperhomocysteinemia (125, 143). This is consistent with data from several experimental studies suggesting that ADMA concentrations in a pathophysiologically high range (3–15 μmol/liter) significantly inhibit vascular NO elaboration (144–146). Recent data from a prospective clinical trial suggest that ADMA is a prognostic marker of cardiovascular and of all-cause mortality in patients with end-stage renal disease (RH Böger & C Zoccali unpublished observation). Future investigations will help to clarify the role of ADMA in pathophysiology and in the therapeutic effects of L-arginine.

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SHORT REPORT

Arginine therapy: a novel strategy to induce nitric oxide production in sickle cell disease

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Summary. To determine the effects of L-arginine (L-Arg) supplementation on nitric oxide metabolite (NO_x) production, oral L-Arg was given to normal controls, sickle cell disease (SCD) patients at steady state and SCD patients hospitalized with a vaso-occlusive crisis (VOC). L-Arg (0·1 g/kg) increased NO_x formation by 18·8 ± 68% in normal controls, whereas steady-state SCD patients demonstrated a paradoxical decrease in NO_x of −16·7 ± 4% ($P = 0·004$). In contrast, patients with VOC demonstrated a dramatic increase in NO_x production by +77·7 ± 103%, a response that was dose dependent. L-Arg appears to be the rate-limiting step in NO_x production during VOC. Oral arginine may therefore benefit SCD patients by inducing an increase in NO production during VOC.

Keywords: nitric oxide, L-arginine, sickle cell disease, vaso-occlusive crisis, nitric oxide metabolites.

Adults with sickle cell disease (SCD) are L-arginine (L-Arg) deficient (Enwonwu *et al*, 1990) and may benefit from supplementation. Our preliminary data suggested that this deficiency may be age dependent and influenced by acute events, with lowest L-Arg levels found during vaso-occlusive crisis (VOC) and acute chest syndrome (ACS) (Morris *et al*, 2000). L-Arg is the precursor to nitric oxide (NO), and NO metabolite (NO_x) levels are also decreased during VOC and ACS (Stuart & Setty, 1999; Morris *et al*, 2000). L-Arg is a safe therapy (Perrine *et al*, 1994) that has demonstrated the capacity to decrease platelet aggregation (Adams *et al*, 1995) and reverse endothelial dysfunction (Lerman *et al*, 1998) in other disease processes and it may represent a new therapeutic intervention in SCD.

NO_x levels were shown to increase after oral L-Arg supplementation in healthy subjects (Kharitonov *et al*, 1995). We undertook a study to determine whether oral L-Arg increases NO_x production in patients with SCD.

MATERIALS AND METHODS

Patients. SCD patients at steady state, patients hospitalized with VOC and non-SCD healthy volunteers ≥ 8 years of age were prospectively enrolled in the study. The study protocol was approved by the Institutional Review Board, and informed consent was obtained for all patients.

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Oral L-Arg was administered during 20 separate occasions to 10 patients with SCD and to four normal controls. Age and sex were similar in all groups.

Mean percentage change in serum NO_x levels was calculated for patients in each category. The mean percentage change represents the mean of the maximum change in NO_x levels from baseline ($t = 0$).

L-Arginine administration. After a fasting period of > 8 h, oral L-Arg (0·5 g/kg, 0·1 g/kg or 0·25 g/kg) was administered to each patient as a one-time dose to determine its impact on NO_x production. Serum L-Arg and plasma NO_x levels were determined at baseline and were followed sequentially over 4 h.

L-Arginine and NO_x measurement. Quantitative plasma L-arginine levels were measured in μmol/l by Quest Diagnostics, San Juan Capistrano, CA, USA.

The formation of NO_x was measured by determination of its stable end products in serum, i.e. nitrite (NO_2) and nitrate (NO_3) in μmol/l, as previously described (Morris *et al*, 2000).

Statistical analysis. Results are expressed as means ± SD. Percent change for NO_x levels are expressed as the mean percentage change ± SD. The paired Student's *t*-test was used to evaluate for significant differences within the same treatment groups, and the Wilcoxon two-sample test was

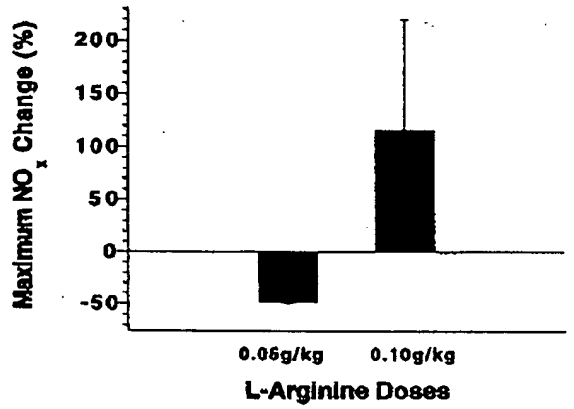

Fig 1. Maximum percent change in nitric oxide metabolite (NO_x) production in sickle cell disease patients with vaso-occlusive crisis after oral L-arginine at low dose (0·05 g/kg, $n = 3$) vs. intermediate dose (0·1 g/kg, $n = 4$).

used to evaluate significant differences between study populations.

RESULTS

Oral L-Arg administration in SCD patients at steady state compared with normal controls

Supplemental L-Arg (0·1 g/kg) was administered to six SCD patients at baseline and four non-SCD normal controls. Baseline L-Arg levels were lower (mean ± SD: 45·5 ± 10·6 vs. 81·2 ± 20·5 μmol/l, $P = 0·006$) and NO_x levels were higher (24·0 ± 8·9 vs. 15·9 ± 10·8 μmol/l respectively) in SCD patients than in normal controls. Serum L-Arg concentrations rose significantly in both groups, peaking at approximately 2 h after arginine administration (144·7 ± 30·3 in SCD patients vs. 164·5 ± 43·4 μmol/l in controls). L-Arg levels decreased more rapidly in the SCD patients by the end of 4 h (90·7 ± 18·3 vs. 142·3 ± 67 μmol/l), but this difference did not reach statistical significance.

Control patients demonstrated a mean increase in endogenous NO_x levels of +18·8 ± 68%. In contrast, NO_x levels decreased in every SCD patient at steady state (−16·7 ± 4%, $P = 0·004$). High-dose L-Arg (0·25 g/kg) elicited a similar paradoxical response, despite differences in peak L-Arg levels (170·7 ± 41·2 vs. 278·0 ± 116·7 μmol/l).

Oral L-Arg administration in SCD patients with VOC

Unlike SCD patients at steady state, patients with VOC underwent a dramatic increase in NO_x production after oral L-Arg (0·1 g/kg, +77·7 ± 103%). This rise in NO_x production occurred despite peak L-Arg levels that were significantly lower in the VOC group than in steady-state patients receiving the same L-Arg dose (93·8 ± 26·6 vs. 170·7 ± 41·8 μmol/l, $P = 0·01$).

Low-dose L-Arg (0·05 g/kg) resulted in a significant decrease in NO_x levels in VOC patients, with a mean percentage change of −48·1 ± 2% (Fig 1), despite achieving similar peak L-Arg levels (87·6 ± 62·2 μmol/l).

Oral L-Arg in the same SCD patient at steady state compared with VOC

One patient was enrolled at steady state and twice during VOC (Fig 2). Oral L-Arg (0·1 g/kg) induced a paradoxical decrease in NO_x levels at steady state, but a significant increase in NO_x production during VOC (maximum percentage change −23·8% vs. +109·4%). NO_x levels decreased, however, during a separate VOC event after low dose (0·05 g/kg) oral L-Arg (−47·7%).

Vital signs, P_{50} and methaemoglobin levels

No changes were noted after oral L-Arg, despite significant alterations in NO_x concentration.

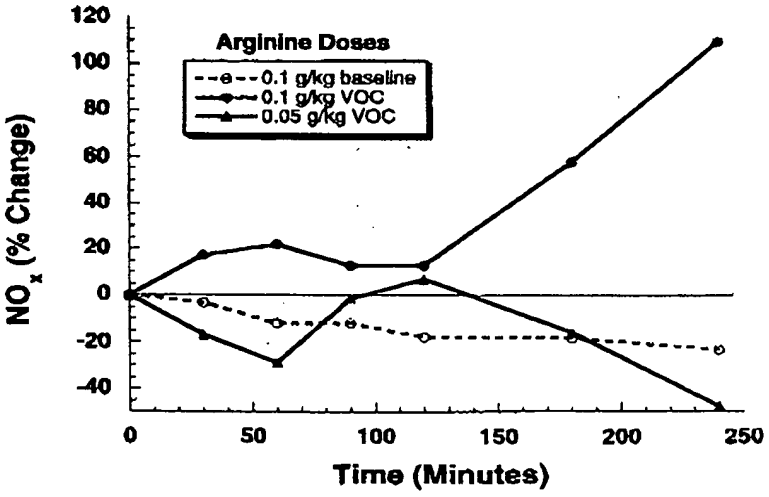

Fig 2. Per cent change in nitric oxide metabolite (NO_x) levels from baseline ($t = 0$) in a representative sickle cell disease patient after oral arginine administration at steady state (0·1 g/kg, no VOC) and during vaso-occlusive crisis (0·05 g/kg VOC and 0·1 g/kg VOC).

DISCUSSION

Previously, the effects of arginine supplementation on NO_x production in SCD patients had not been reported. The increased metabolism of oral L-Arg in SCD patients compared with normal controls is accelerated even further during VOC. An increased compensatory demand for NO in SCD may lead to depletion of available L-Arg stores, resulting in decreased L-Arg and NO_x levels during VOC (Morris *et al*, 2000). If an acute L-Arg deficiency during VOC was the cause of low NO_x levels, then L-Arg administration would be expected to increase NO_x production. Our data suggest that a threshold L-Arg concentration may be necessary during VOC to induce NO_x production. Supplementation with the lower L-Arg dose (0·05 g/kg) during VOC possibly provided enough substrate to activate the NO-producing enzyme nitric oxide synthase (NOS) but was insufficient to sustain increases in NO_x production, ultimately resulting in decreased NO_x levels. Previous investigations have shown that L-Arg concentration influences what is produced by NOS, synthesizing superoxide in lieu of NO when L-Arg concentrations are low. (Xia *et al*, 1996). As superoxide rapidly converts NO to other reactive nitric oxide species (Xia *et al*, 1996) not measured by our study, this may be an explanation for the paradoxical drop in NO_x found in SCD patients at steady state and after insufficient L-Arg dosing in VOC. The duplication of these patterns of NO_x production demonstrated within a single patient at steady state and VOC supports this hypothesis.

Even with our small sample size, trends in NO_x production were consistently different in VOC patients from patients at steady state. Although low-dose L-Arg was subtherapeutic, lower doses may become therapeutic after repeated supplementation and restoration of depleted arginine stores.

Whether increased NO_x production will have a favourable impact on VOC remains to be determined, particularly in light of the deleterious potential of nitric oxide (Xia *et al*, 1996). Yet, low NO_x levels during VOC and ACS (Stuart & Setty, 1999; Morris *et al*, 2000) and an inverse relationship between pain scores and NO_x levels in SCD patients with VOC (Lopez *et al*, 1996) support an association between low NO_x levels and vaso-occlusion.

Recent studies demonstrating NO binding to haemoglobin (Gladwin *et al*, 1999) implicate another mechanism of action for NO in the regulation of vascular tone. Inhaled NO has been advocated as a possible therapy for ACS (Atz & Wessel, 1997), and clinical trials of inhaled NO in patients with acute respiratory distress syndrome are ongoing (Dellinger *et al*, 1998). Yet the delivery of inhaled NO is cumbersome and not without potential side-effects that require close monitoring in an acute care setting, limiting its application. L-Arginine, on the other hand, is a safer and more easily administered alternative to inhaled NO and may have significant therapeutic potential.

Arginine therapy may be a novel strategy to improve VOC by inducing nitric oxide production in SCD; however, a controlled clinical trial assessing the safety and efficacy of oral L-Arg is necessary before any definitive recommendations can be made.

ACKNOWLEDGMENTS

We would like to thank Lynne Neumayr for her assistance with statistical analysis. This study was supported in part by the National Institute of Health grants RR01271-19, Paediatric Clinical Research Center and HL-20985.

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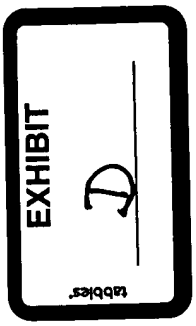
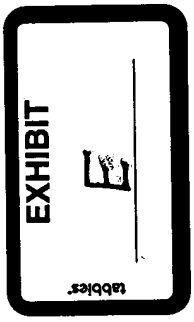
S-nitrosothiols in humans.

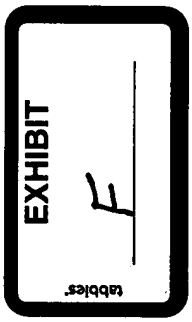
1. Lees C, Langford E, Brown AS, de Belder A, Pickles A, Martin JF, Campbell S. The effects of S-nitrosoglutathione on platelet activation, hypertension, and uterine and fetal Doppler in severe preeclampsia. Obstet Gynecol.88:14-9 1996. OBJECTIVE: To determine the effects of the platelet-specific nitric oxide donor S-nitrosoglutathione on women with severe preeclampsia. METHODS: Ten women with severe preeclampsia or preeclampsia with severe fetal compromise at 21-33 weeks' gestation each received a 60-90-minute intravenous infusion of 50-250 micrograms/minute of S-nitrosoglutathione. Each was hypertensive, despite conventional oral antihypertensive therapy in eight. Maternal blood pressure, heart rate, platelet activation, uterine artery, and fetal Doppler indices were measured during the infusion. RESULTS: A dose-dependent reduction in mean arterial pressure from 125 mmHg (95% confidence interval [CI] 117-133) to 103.5 (95% CI 97-111) ($P < .005$) and an increase in pulse rate from 73.7 beats per minute (95% CI 64.3-84.5) to 89.1 (95% CI 81.2-97.8) ($P < .02$) was observed during the infusion. Mean uterine artery resistance index fell from 0.76 (95% CI 0.73-0.81) to 0.70 (95% CI 0.65-0.75) ($P < .009$). Platelet activation measured by P-selectin expression was reduced from 3.02% (95% CI 2.09-4.36) to 1.22% (95% CI 0.94-1.58) ($P < .01$). Fetal Doppler indices (umbilical artery, middle cerebral artery, and thoracic aorta) showed no significant changes during the infusion. CONCLUSION: S-nitrosoglutathione infusion reduced material mean arterial pressure, platelet activation, and uterine artery resistance without further compromising fetal Doppler indices. This study suggests that platelet-specific nitric oxide donors may prove beneficial in the management of severe preeclampsia.



2. Molloy J, Martin JF, Baskerville PA, Fraser SC, Markus HS. S-nitrosoglutathione reduces the rate of embolization in humans. Circulation.98:1372-5 1998. BACKGROUND: Antiplatelet agents presently used in the secondary prevention of cardiovascular disease fail to prevent the majority of cases of recurrent stroke and systemic embolization. An evaluation of the efficacy of new agents is hampered by a lack of in vivo models in humans. Asymptomatic cerebral embolic signals (ES) may be detected with the use of transcranial Doppler ultrasonography. These signals are particularly common after carotid endarterectomy, and this provides a situation in which new antiplatelet agents can be evaluated. With this model, we determined the effectiveness of S-nitrosoglutathione (GSNO), a nitric oxide donor with relative platelet specificity, in reducing cerebral embolization. METHODS and RESULTS: Transcranial Doppler ultrasound recordings from the ipsilateral middle cerebral artery were made after carotid endarterectomy in 12 control patients and 12 patients receiving intravenous GSNO from the induction of anesthesia until 2 hours after skin closure. Recording times were 0.5 to 3.5, 6 to 7, and 24 to 25 hours after skin closure. The Doppler signal was recorded onto tape, and analysis for ES was performed, with the investigators blinded to treatment group. All patients received aspirin 300 mg/d before surgery and 5000 IU of heparin during surgery. The median (range) number of ES detected during the initial 3-hour postoperative recording was markedly reduced in the GSNO group compared with the control group: 7.5 (0 to 61) versus 38.5 (1 to 219) ($P=0.018$). This difference persisted until 6 hours after surgery. CONCLUSIONS: Despite the administration of aspirin and heparin, frequent embolization occurred and was markedly reduced after the administration of GSNO. This demonstrates the potential use of platelet-specific nitric oxide donors in the treatment of thromboembolic disease. This model of cerebral embolism may allow determination of the effectiveness of new antiplatelet agents in humans.

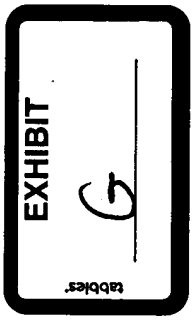


3. Kuo YR, Wang FS, Jeng SF, Lutz BS, Huang HC, Yang KD. Nitrosoglutathione improves blood perfusion and flap survival by suppressing iNOS but protecting eNOS expression in the flap vessels after ischemia/reperfusion injury. Surgery.135:437-46. 2004. BACKGROUND: The effects of nitric oxide (NO) on the microcirculation and free tissue survival remain controversial. With the use of a rat inferior epigastric artery flap as an ischemia/reperfusion injury (I/R) model, we investigated whether exogenous NO donation regulates endogenous NO synthase (NOS) expression in the flap vessels and promotes flap survival. METHODS: Thirty minutes before flap reperfusion, normal saline (1 ml), nitrosoglutathione (GSNO 0.2, 0.6, 3 mg/kg), or N(G)-nitro-L-arginine-methyl ester (L-NAME, 450 mg/kg), was injected intravenously into 20 rats. Total plasma NOx (NO(2)-/NO(3)-) was measured to reflect NO production. Immunohistochemical staining was investigated for the endothelin-1 (ET-1) and NOS isoforms expression on the flap vessels. NOS isoforms expression was evaluated by Western

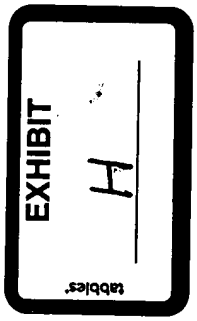


blot. Laser-Doppler flowmetry monitored flap perfusion. Survival areas were assessed by gross examination at 7 days postoperatively. RESULTS: Flap ischemia at 12 hours followed by reperfusion resulted in endothelial cell damage, as demonstrated by induction of iNOS and ET-1 expression in the flap vessels. An optimal dose of nitrosoglutathione (0.6 mg GSNO/kg) significantly increased plasma NOx levels (P=.027) and improved flap perfusion by laser Doppler measurement (P=.014), and increased the flap viability area (P<.001). Additionally, it selectively suppressed iNOS induction, but enhanced eNOS expression and decreased ET-1 deposition in the flap vessels. In contrast, an NOS inhibitor, N(G)-nitro-L-arginine methyl ester, inhibited both iNOS and eNOS expression in the flap vessels, decreased endogenous NOx production, and compromised flap viability. CONCLUSION: This study indicates that intravenous administration of exogenous GSNO can appropriately donate NO to suppress iNOS induction and enhance eNOS expression in pedicle vessels, resulting in better blood perfusion and a higher flap survival after I/R injury.

4. Snyder AH, McPherson ME, Hunt JF, Johnson M, Stamler JS, Gaston B. Acute effects of aerosolized S-nitrosoglutathione in cystic fibrosis. Am J Respir Crit Care Med.165:922-6 2002. S-nitrosoglutathione (GSNO), a naturally occurring constituent of airway lining fluid, enhances ciliary motility, relaxes airway smooth muscle, inhibits airway epithelial amiloride-sensitive sodium transport, and prevents pathogen replication. Remarkably, airway levels of GSNO are low in patients with cystic fibrosis (CF). We hypothesized that replacement of airway GSNO would improve gas exchange in CF. In a double-blind, placebo controlled study, we administered 0.05 ml/kg of 10 mM GSNO or phosphate buffered saline by aerosol to patients with CF and followed oxygen saturation, spirometry, respiratory rate, blood pressure, heart rate, and expired nitric oxide (NO). Nine patients received GSNO and 11 placebo. GSNO inhalation was associated with a modest but sustained increase in oxygen saturation at all time points. Expired NO increased in the low ppb range with GSNO treatment, peaking at 5 minutes but remaining above baseline at 30 minutes. There were no adverse effects. We conclude that GSNO is well tolerated in patients with CF and improves oxygenation through a mechanism that may be independent of free NO. Further, GSNO breakdown increases expired NO. We suggest that therapy aimed at restoring endogenous GSNO levels in the CF airway may merit study.



5. Rassaf T, Kleinbongard P, Preik M, Dejam A, Gharini P, Lauer T, Erckenbrecht J, Duschin A, Schulz R, Heusch G, Feelisch M, Kelm M. Plasma nitrosothiols contribute to the systemic vasodilator effects of intravenously applied NO: experimental and clinical Study on the fate of NO in human blood. Circ Res.91:470-7 2002. Higher doses of inhaled NO exert effects beyond the pulmonary circulation. How such extrapulmonary effects can be reconciled with the presumed short half-life of NO in the blood is unclear. Whereas erythrocytes have been suggested to participate in NO transport, the exact role of plasma in NO delivery in humans is not clear. Therefore, we investigated potential routes of NO decomposition and transport in human plasma. NO consumption in plasma was accompanied by a concentration-dependent increase in nitrite and S-nitrosothiols (RSNOs), with no apparent saturation limit up to 200 micro mol/L. The presence of red blood cells reduced the formation of plasma RSNOs. Intravenous infusion of 30 micro mol/min NO in healthy volunteers increased plasma levels of RSNOs and induced systemic hemodynamic effects at the level of both conduit and resistance vessels, as reflected by dilator responses in the brachial artery and forearm microvasculature. Intravenous application of S-nitrosoglutathione, a potential carrier of bioactive NO, mimicked the vascular effects of NO, whereas nitrite and nitrate were inactive. Changes in plasma nitrosothiols were correlated with vasodilator effects after intravenous application of S-nitrosoglutathione and NO. These findings demonstrate that in humans the pharmacological delivery of NO solutions results in the transport and delivery of NO as RSNOs along the vascular tree.



compared; therefore, data were analyzed separately for HbS and HbAA genetic backgrounds. High-IL10 genotype compared with low-IL10 genotype was significantly associated with a lower risk for parasitemia (RR.87, 95% CI.78-.96, p <0.05) among children with normal Hb (HbAA) genotype but not in children with HbS genotype High-IL10 genotype was associated with a reduced risk (RR.67, 95% CI.45-1.01, p=.053) for malaria anemia as compared with low-IL10 genotype irrespective of HbS status. These findings suggest that high-IL10 genotype protects against *P. falciparum* infection in children with normal HbAA genotype and protects against malaria-associated anemia in children with both HbS and HbAA genetic backgrounds.

514 THE PHENOTYPE OF CD8+ T CELLS THAT FUNCTION IN THE PATHOGENESIS OF EXPERIMENTAL CEREBRAL MALARIA. Yanez DM, LaFleur G, Loveland LM, Weidanz WP. Department of Medical Microbiology and Immunology, University of Wisconsin Medical School, UW-Madison, Madison, WI.

Our observation that CD8+ T cells, in addition to other lymphocyte populations are essential for the pathogenesis of experimental cerebral malaria (CM) led us to examine their activation status and costimulatory requirements during *Plasmodium berghei* ANKA (PbA) infection. Cytofluorimetric analysis revealed that CD8+ T cells isolated from the brains of mice dying with CM were increased in both size and granularity and expressed $CD44_{high}$ and $CD69_{+}$ surface markers, indicating that that these cells were activated. Because CD28 and CD40L (CD154) are costimulatory molecules that function in the activation, proliferation, and prolonged survival of T cells, we examined the requirements for these molecules in the pathogenesis of CM by using gene-targeted knock-out (KO) mice. When KO mice deficient in either CD40L or CD28 were infected with PbA, only mice lacking CD40L failed to develop disease suggesting that CD40L, but not CD28 plays an essential role in CM. Although CD28 is involved in T cells activation, T cells may be activated by other co-stimulatory molecules (e.g. CD40) in CD28-deficient mice because these mice developed CM. The necessity for CD40L in the generation of CM may reflect its necessary role in the generation of appropriate Th1 and CTL responses. Many activated CD8+ T cells in brains from CM-positive mice utilized Vβ 8.1, 2_{+}. Results of antibody depletion studies by others indicate that Vβ 8.1, 2_{+} T cells mediate CM pathogenesis, our data suggests further that these activated Vβ 8.1, 2_{+} CD8+ T cells may function in the brain to cause CM pathology. Moreover, cerebral CD8+ T cells stained $CD44_{high}$ and $CD62L_{neg}$, thus exhibiting the phenotype of a distinct population of pathogenic T cells that infiltrate the brain during certain inflammatory and autoimmune diseases. Collectively, these data suggest the presence of a potentially pathogenic CD8+ T cell subset in the brains of mice with PbA-induced CM.

515 HIGHER RATIO OF PLASMA TNF:MONONUCLEAR CELL NITRIC OXIDE SYNTHASE (NOS) ACTIVITY IN ACUTE VIVAX VS FALCIPARUM MALARIA: A CORRELATE OF THE LOWER FEVER THRESHOLD IN *PLASMODIUM VIVAX* INFECTION? Anstey NM, Tjitra E, Maniboey H, Misukonis MA, Solihin A, Baird JK, Fryauff, D, Hobbs M, Granger DL, Weinberg JB. International Health Unit, Menzies School of Health Research, Darwin, NT, Australia; National Institute of Health Research and Development, Ministry of Health, Jakarta, Indonesia; Menzies School of Health Research, Jayapura, Papua, Indonesia; Division of Hematology-Oncology, Duke University and VA Medical Centers, Durham, NC; US NAMRU-2, Jakarta, Indonesia; Division of Infectious Diseases, University of Utah, UT.

It has long been noted that infection with *Plasmodium vivax* causes fever at a lower level of parasitemia than infection with *P. falciparum*. TNF is a major mediator of fever in malaria. In contrast, nitric oxide is thought to be antipyretic through its ability to downregulate fever-inducing cytokines such as TNF. We hypothesised that relative to falciparum malaria, mononuclear cell NOS activity would be lower and plasma TNF levels higher in symptomatic vivax malaria. In studies in Jayapura, Papua, Indonesia, we compared plasma TNF levels, blood mononuclear cell NOS activity and the TNF:NOS ratio in 58 patients with symptomatic, uncomplicated falciparum malaria and 45 with symptomatic vivax malaria. Although parasitemia was higher in falciparum malaria (geometric mean 3760/ul vs 1642/ul), TNF levels were higher in vivax malaria (geometric mean 1019 pg/ml [95% CI 640-1622] vs 612 pg/ml [95% CI 394-951]). In contrast, blood mononuclear cell (PBMC) NOS activity (L-arginine to L-citrulline conversion) was suppressed to a lower level in vivax than falciparum malaria (geom mean 2081 pmol/mg [95% CI 1731-2501] vs 2486 pmol/mg [95% CI 2212-2794]), with each being lower than basal NOS activity found in uninfected adults in this area (geometric mean 3209 pmol/mg). The TNF:NOS ratio was significantly higher in vivax (geometric mean 0.58 [95%CI 0.33-0.99)] than falciparum malaria (geom. mean 0.25 [95%CI 0.16-0.38]); p=0.048 in a model controlling for parasitemia and ethnic group. Results suggest that acute symptomatic infection with *P. vivax* suppresses mononuclear cell NOS activity and induces a TNF

response to a greater extent than acute infection with *P. falciparum*. This may account for the lower fever threshold in vivax malaria. Results are consistent with the hypothesis that the toxin(s) of *P vivax* is a more potent modulator of the TNF/NOS response than that of *P. falciparum*.

516 CHEMOKINES IN PLACENTAL MALARIA. Abrams EA, Brown H, Chensue SW, Turner GDH, Tadesse E, Lema VM, Molyneux ME, Meshnick SR, Rogerson SJ. Department of Anthropology, University of Michigan, Ann Arbor MI; Nuffield Department of Clinical Laboratory Sciences, John Radcliffe Hospital, Oxford UK; Department of Pathology, University of Michigan, Ann Arbor MI; Department of Obstetrics and Gynaecology, College of Medicine, University of Mlawi, Blantyre Malawi; Malawi-Liverpool-Wellcome Trust Clinical Research Programme, College of Medicine, University of Malawi, Blantyre Malawi; Department of Epidemiology, University of North Carolina, Chapel Hill NC; Department of Medicine, Royal Melbourne Hospital, University of Melbourne, Australia.

Plasmodium falciparum malaria during pregnancy is associated with poor birth outcomes, particularly low birth weight and maternal anemia. We and others have recently described associations between hemozoin-containing placental intervillous monocytes and low birth weight and maternal hemoglobin concentrations. In exploring the mechanisms by which monocytes are recruited to the placenta, we have examined the role of placental chemokines. Chemokines are small polypeptides which induce migration of leucocytes to sites of inflammation. CC, or beta -chemokines, function primarily as monocyte attractants, and CXC or alpha-chemokines act as neutrophil attractants. In this study, we used ribonuclease protection assay of whole placental tissue to quantitate placental chemokine mRNA expression, and measured placental plasma protein levels by ELISA, in women with and without placental malaria infection at delivery. Immunohistochemistry was used to localise production to maternal or fetal placental compartments. mRNA expression of the beta chemokines macrophage inflammatory protein-1 alpha (MIP-1a), MIP-1b, monocyte chemoattractant protein-1 (MCP-1), and I-309 was significantly higher in the presence of malaria infection and correlated strongly with monocyte density in the placental intervillous space. mRNA expression of the Alpha-chemokine interleukin-8 (IL-8) was also associated with placental monocyte density, whereas RANTES, IP-10 and lymphotactin expression did not alter with malaria or monocyte counts. HIV status and mode of delivery were not associated with mRNA levels. Placental plasma concentrations of MIP-1a, MIP-b, and IL-8 were increased in women with placental malaria, especially those with accompanying monocyte infiltrates. IL-8 concentrations were negatively correlated with infant birth weights. By immunohistochemistry, MCP-1 production was localised to fetal stromal cells and MIP-1b to some but not all hemozoin-laden maternal macrophages. Local placental production of chemokines is increased in malaria, and may be an important trigger for monocyte accumulation in the placenta.

517 SIGNATURE PATTERNS OF GLOBAL GENE EXPRESSION IN HUMAN MALARIA INFECTION USING HIGH-DENSITY OLIGONUCLEOTIDE MICROARRAYS. Ockenhouse CF, Kester KE, Cummings J, Stewart, A, Nau M, Vahey M. Department of Immunology, Walter Reed Army Institute of Research, Silver Spring, MD; Division of Retrovirology, Walter Reed Army Institute of Research, Silver Spring, MD.

The explosion of information coming from the human genome project promises to transform the nature of investigations of host-parasite relationships pertaining to the diagnosis, pathogenesis, and immunology of infectious disease. In this study we explore how early *Plasmodium falciparum* infection in human volunteers experimentally infected by mosquito challenge influences transcriptional regulation of thousands of human genes early in the course of infection. We applied a functional genomic screen of >7000 human genes on Affymetrix GeneChip oligonucleotide microarrays using mRNA prepared from peripheral blood mononuclear cells (PBMCs) collected from volunteers at various stages of infection. Two sequential studies on 11 volunteers each (n = 22) provided a highly reproducible And powerful statistical basis for examination of genes induced and repressed during malaria infection. The PBMC is a potent biological sensor for infection that occurs not only in the peripheral circulation (blood-stage malaria) but also detects and responds to infection in anatomic sites inaccessible to normal sampling (liver-stage malaria). Using a variety of bioinformatic tools including standard paired t-test, hierarchical clustering, principal component analysis, self-organizing maps, and artificial neural networks, we discovered new and novel biological processes in inflammation, signal transduction, transcriptional regulation, innate immunity, and adaptive immunity. We will present data showing hundreds of genes induced and repressed during liver stage malaria which are shared or unique with those differentially regulated during early blood stage infection. In addition, a global examination of those genes regulated by